



Effect of Ethanol Leaf Extract of *Lantana camara* on Fasting Blood Glucose, Body Weight and Kidney Function Indices of Alloxan-Induced Diabetic Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study was aimed at investigating the effect of ethanol leaf extract of *Lantana camara* on the fasting blood glucose, body weight and kidney function indices of alloxan-induced diabetic albino Wistar rats. Thirty male albino Wistar rats of weight 100-181 g were randomly assigned six groups

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of five rats each. Group 1 served as normal control. Group 2-6 animals were induced with 150 mg/kg body weight (B.W) of alloxan monohydrate intraperitoneally. Fasting blood glucose was checked 72hrs after induction and animals with blood glucose 200mg/dl and above were considered diabetic. Group 3 was treated with 50 mg/kg B.W of glibenclamide. Group 4,5 and 6 were orally treated with 600, 800 and 1000 mg/kg B.W of *Lantana camara* extract respectively for 21days. Blood sample was obtained by tail puncture to check glucose levels weekly with the use of glucometer. Animals treated with 600, 800 and 1000 mg/kg B.W of the extract showed significant decrease ($P<0.05$) in blood glucose, creatinine, urea and uric acid compared to untreated diabetic animals. Body weight of treated groups increased significantly ($P<0.05$) compared to the untreated group. The results obtained were synonymous to the glibenclamide treated group. This suggests that the extract possess antidiabetic potential and could be used in the management of diabetes.

Keywords: Diabetes; *Lantana camara*; alloxan; glucose; kidney function.

1. INTRODUCTION

Diabetes mellitus is a pancreatic metabolic disorder that results in the alteration of glucose metabolism due to irregularities in insulin secretion or inaction of insulin to glucose [1]. "The condition is characterized by frequent urination, increased thirst, hyperglycemia. Diabetes mellitus has several aetiological progressions ranging from autoimmune destruction of β -cells and subsequent insulin deficiency (Type I) or decline in insulin activity (Type II)" [2]. However, "the two types of diabetes aggravate the alteration in carbohydrates, fat and proteins metabolism resulting in microvascular and macrovascular complications" [3]. "Other diabetes mellitus complications includes but not limited to nephropathy which culminates to dysfunction of renal function architecture, retinopathy with possible vision loss, non-alcohol fatty liver disease, cardiomyopathy, neuropathy and hepatocellular carcinomas" [4]. Diabetes Mellitus (DM) is recognized as one of the leading cause of morbidity and mortality. According to [5], the incidence of diabetes may globally rise by 48% between 2017 – 2045. Therefore, the regulation of blood glucose is a paramount strategy in abating hyperglycemia and its associated complications as well as improving the life of diabetic patients [6].

"The administration of hypoglycemic agents is currently in practice to cushion the hyperglycemic effect but these chemotherapeutics possess adverse effects upon continuous usage" [7]. "Currently, scientific investigations and clinical evaluations have confirmed the potential applications of medicinal plants and herbal formulations in the restoration of normal glucose homeostasis. The World Health Organization (WHO) estimates that 80% of individuals in

developing nations particularly Africa use traditional medicines for the treatment of different diseases" [8].

Lantana camara is an important medicinal plant belonging to the family verbenaceae. Pharmacologically, *Lantana camara* is documented to possess hepatoprotective, antimicrobial, antioxidant, antidiabetic effects etc. [9]. "*Lantana camara* is a flowering plant with possible use in modern medicine. Phytochemical evaluation of *Lantana camara* leaves revealed the presence of some bioactive compounds e.g flavonoids, phenolic compounds, alkaloids, saponins, glycosides tannins etc" [10]. The study was aimed at investigating scientifically the effect of ethanol leaves extract of *Lantana camara* on the fasting blood glucose level, body weight and kidney function indices of alloxan-induced diabetic albino Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection of Preparation of Plant Extract

"*Lantana camara* leaves were collected from a farmland in Ikot Osurua, Ikot Ekpene Local Government Area, Akwa Ibom State. The leaves were identified and authenticated by a Botanist in the Department of Biological Sciences, Akwa Ibom State Polytechnic, Ikot Osurua. Fresh leaves of *Lantana camara* were detached from the stems, sorted, rinsed with distilled water, sliced and dried under shade. The dried leaves were ground into fine powder using an electric blender. Thereafter, 110 g of the powder was extracted in 70% ethanol by stirring intermittently, macerated for 72 hours and filtered through Whatman No. 4 filter paper. The filtrate was concentrated to paste by heating in a water bath at 40 °C and stored at 4 °C for the analysis" [11].

2.2 Induction of Diabetes

“30 albino Wistar rats weighing 100 – 181 g were obtained from the animal house of Department of Biological Science, Akwa Ibom State Polytechnic, Ikot Osurua. The animals were fed *ad libitum* with commercial feed and clean drinking water; and acclimatized for two weeks before experiment. The rats were divided into non-diabetic control and diabetic groups” [11].

“Diabetes was induced in the experimental rats by intraperitoneal administration of 150 mg/kg body weight of alloxan monohydrates dissolved in 0.9% saline after overnight fast. After induction all rats were allowed free access to feed and clean water. 72 hours after induction, blood sample obtained through the tail tip puncture of the rats were used to confirm diabetes in the rats by testing for hyperglycemia using glucometer. Albino Wistar rats with fasting blood glucose concentration of 200mg/dl and above were considered diabetic and selected for the experiment. The use of albino Wistar rats for the study was carried out in accordance with guidelines set by Institute for Laboratory Animal Research” [12].

2.3 Experimental Design

The 30 albino rats were divided into 6 groups of 5 rats each.

- Group 1 - Normal Control Group
- Group 2 - Diabetic untreated group
- Group 3 - Diabetic treated group (received 5 mg/kg body weight glibenclamide).
- Group 4 - Diabetic rats received 600 mg/kg body weight of *Lantana camara*
- Group 5 - Diabetic rats received 800 mg/kg body weight of *Lantana camara*
- Group 6 - Diabetic rats received 1000 mg/kg body weight of *Lantana camara*

Treatment was administered once a day orally for 21 days.

2.4 Determination of Blood Glucose Concentration

Fasting blood glucose was determined five times in the course of the study. Firstly, before the induction of diabetes by alloxan. Secondly, after 72 hours of induction of diabetes. The measurement was continued first week, second and third week of treatment. The blood glucose levels were taken by sterilizing the tails of the

albino Wistar rats with 10% alcohol, punctured with needles and the exuded blood was allowed to touch the test strip which was inserted into a calibrated glucometer (fine test glucometer). This gave direct reading after 9 seconds in mg/dL.

2.5 Measurement of Body Weight

The body weight of the animals were tabulated five times during the study (i.e. before alloxan injection (baseline values) 72 hours after alloxan injection, first week, second week and third week of the treatment period) with an ordinal weighing scale throughout the 21 day treatment.

2.6 Sample Collection for Analysis

At the end of 21 days treatment, the rats were made to fast overnight and then euthanized under chloroform vapour and sacrificed. Whole blood was obtained by cardiac puncture into non-heparinized tubes and were allowed to clot for 1 hour 30 minutes. The sample was then centrifuged at 4000rpm for 30 minutes to recover the serum for the various biochemical assays.

2.7 Biochemical Assays

2.7.1 Determination of serum urea

This was carried out using the method of [13]. Standard commercial kit supplied by Randox was used. Ten microliters (10µl) each of standard and serum sample were pipetted into respective test tubes labelled standard and sample. Then one thousand microliters (1000 µl) of working reagent was added to each test tube labelled reagent blank, standard and sample and mixed. After 30 seconds, initial absorbance was read simultaneously. Again, absorbance was read after 1, 2 and 3 minutes at 340 nm.

Calculation:

$$\text{Urea concentration} = (\Delta A_{\text{sample}} / \Delta A_{\text{standard}}) \times \text{standard concentration}$$

2.7.2 Determination of serum creatinine

This was carried out using the method of [14]. Standard commercial kit supplied by Randox was used. Fifty microliters (50 µl) each of distilled water, standard and serum sample were pipetted into respective test tubes labelled reagent blank, standard and sample. Then Five hundred microliters (500 µl) of working reagent was

added to each test tube and mixed. After 30 seconds, absorbance A_1 of the standard and sample was read. Exactly 2 minutes later, absorbance A_2 of standard and sample was read at 492 nm.

Calculation:

$$\text{Creatinine concentration} = (\Delta A_{\text{sample}} / \Delta A_{\text{standard}}) \times \text{standard concentration}$$

2.7.3 Determination of serum uric acid

This was carried out using the method of [15]. Standard commercial kit supplied by Beacon was used. One thousand microliters (1000 μ l) each of enzyme reagent was pipetted into respective test tubes labelled reagent blank, standard and sample. Then twenty-five microliters (25 μ l) of standard and sample were added to the respective test tubes mixed well and incubated at 37°C for 5 minutes. Then, absorbance of the standard and sample were measured against reagent blank at 505 nm.

Calculation:

$$\text{Urea concentration} = (A_{\text{sample}} / A_{\text{standard}}) \times \text{standard concentration}$$

2.8 Statistical Analysis

Data analysis was performed using minitab statistical package. Values were expressed as mean \pm standard error of mean (SEM). Statistical significance of the results between groups were determined using ANOVA.

Differences between means were considered significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

Diabetes is a metabolic alteration in blood glucose homeostasis due to insulin insufficiency, insensitivity or both. The control of glucose levels within the physiological milieu is necessary for effective functioning of the biological system. However, alteration of this biomolecule as a result of metabolic defects can lead to metabolic dysfunction. Alloxan is a diabetogenic agent that its mechanism of action is based on the destruction of the pancreatic beta cells through the generation of reactive oxygen species [15]. Uncontrolled diabetes is one of the predisposing factors to kidney dysfunction [16].

The study showed a non-significant difference in the fasting blood glucose of all the animals prior to induction of diabetes. However, post-diabetic induction indicated a significant increase in the fasting blood glucose of all the groups when compared to the normal control. Following 21 days treatment with leaf extract, the fasting blood glucose level decreased significantly when compared to the untreated diabetic group. These observations are in line with the report of [17]. The report also corroborates with the report of [18] who studied the antidiabetic activity of *Lantana camara* fruit in normal and streptozotocin-induced diabetic rats. The study also aligns with the report of [19] who documented that triterpenoid glycoside in *Lantana camara* exerted sugar lowering property in streptozotocin induced diabetic rats. The increased in the glucose levels after diabetic induction suggest the destruction of pancreatic β -cells resulting in insensitivity of insulin receptor to insulin or loss of the efficacy of the pancreas to secrete insulin required for glucose absorption [20].

The reduction in blood glucose level of the animals treated with ethanol leaf extract of *Lantana camara* may be due to renewed β -cells and or increased sensitivity to insulin, activated by the constituent(s) of the plant extract [21]. The mechanism of hypoglycemic effect of the extract may be attributed to increased insulin sensitivity and upregulation of insulin receptors or short activation of the beta-cells of the pancreas resulting to insulin release [22]. Furthermore, the hypoglycemic activity of this plant could be a resultant effect of the inhibition of hepatic glucose synthesis or activation of glucose utilization by peripheral tissues as well as inhibitors to tubular renal glucose re-absorption [23]. It was observed that 1000 mg/kg bw of *L. camara* extract administration reduced blood glucose comparable to glibenclamide. This observation also conformed to the report of [24,25,26].

Bodyweight evaluation is one of the general indicators employed in assessing the metabolic regulation for diabetes mellitus. The study indicated a significant reduction in bodyweight of untreated diabetic group when compared to the normal. The observation is similar to the report of [27]. The decrease in bodyweight may follow the fact that diabetes mellitus is associated with increased glycogenolysis, lipolysis and gluconeogenesis which result in muscle wasting and excessive breakdown of tissue proteins [28].

Table 1. Effect of ethanol leaf extract of *Lantana camara* on the fasting blood glucose of alloxan-induced diabetic rats

Groups	Blood glucose Before Induction (mg/dl)	Blood glucose 72 hours after Induction	Blood glucose After 1 Week treatment	Blood glucose After 2 Weeks treatment	Blood glucose After 3 Weeks treatment
1	93.60 ± 2.66 ^{a,b}	80.20 ± 3.58 ^b	86.40 ± 3.96 ^c	85.80 ± 3.10 ^c	88.80 ± 6.41 ^b
2	86.80 ± 5.14 ^{a,b}	247.80 ± 13.90 ^a	225.40 ± 16.50 ^a	213.20 ± 3.43 ^a	211.20 ± 2.71 ^a
3	102.60 ± 3.31 ^a	213.80 ± 5.50 ^a	153.40 ± 17.40 ^b	150.60 ± 11.6 ^b	82.20 ± 2.75 ^b
4	79.60 ± 2.01 ^b	215.80 ± 4.44 ^a	174.20 ± 11.40 ^{a,b}	160.80 ± 10.3b ^a	109.40 ± 10.00 ^b
5	91.80 ± 4.80 ^{a,b}	206.80 ± 14.7 ^a	165.60 ± 16.40 ^b	148.20 ± 3.43 ^b	100.60 ± 7.63 ^b
6	81.20 ± 3.12 ^b	251.80 ± 20.7 ^a	174.80 ± 8.89 ^{a,b}	133.00 ± 3.39 ^b	90.40 ± 6.48 ^b

Values with different superscripts are significantly different @ $P < 0.05$ and are expressed as Mean ± SEM

Table 2. Effect of ethanol leaf extract of *Lantana camara* on the Bodyweight (g) of alloxan-induced diabetic rats

Groups	Body weight Before Induction (mg/dl)	Body weight 72 hours after Induction	Body weight After 1 Week treatment	Body weight After 2 Weeks treatment	Body weight After 3 Weeks treatment
1	100.00 ± 6.63 ^c	108.00 ± 5.91 ^b	116.6 ± 7.48 ^b	107.00 ± 2.61 ^{b,c}	135.6 ± 13.0 ^{a,b}
2	156.20 ± 4.85 ^{a,b}	142.80 ± 4.03	141.4 ± 8.02 ^{a,b}	143.02 ± 2.61 ^a	112.90 ± 1.95 ^{a,b}
3	181.80 ± 6.66 ^a	150.60 ± 12.2	149.20 ± 3.40 ^{a,b}	149.12 ± 4.31 ^a	164.16 ± 9.49 ^a
4	131.20 ± 5.42 ^{b,c}	122.00 ± 4.34 ^a	156.80 ± 9.26 ^a	122.59 ± 3.80 ^b	146.82 ± 1.35 ^{a,b}
5	145.20 ± 9.72 ^{a,b,c}	100.60 ± 7.63	139.2 ± 9.19 ^{a,b}	101.86 ± 4.17 ^c	156.10 ± 2.25 ^b
6	153.80 ± 6.22 ^{a,b}	98.40 ± 4.51	156.80 ± 8.97 ^a	100.20 ± 5.08 ^c	156.00 ± 1.66 ^{b,c}

Values with different superscript are statistically significant @ $P < 0.05$ N = 5. Values are expressed as mean ± SEM

Table 3. Effect of ethanol leaf extract of *Lantana camara* on kidney function indices of alloxan-induced diabetic rats

Groups	Urea (mmol/L)	Creatinine (mmol/L)	Uric acid (mg/dL)
1	7.06 ± 0.29 ^b	46.40 ± 9.35 ^b	0.07 ± 0.01 ^b
2	9.57 ± 0.65 ^a	57.80 ± 3.02 ^a	0.13 ± 0.05 ^c
3	7.34 ± 0.98 ^b	51.00 ± 1.77 ^b	0.12 ± 0.01 ^b
4	8.08 ± 1.12 ^b	54.40 ± 2.91 ^{a,b}	0.14 ± 0.05 ^{a,b,c}
5	7.60 ± 0.65 ^b	51.80 ± 4.18 ^b	0.15 ± 0.01 ^{a,b,c}
6	7.48 ± 0.31 ^b	50.60 ± 2.48 ^b	0.07 ± 0.01 ^b

Values with different superscript are statistically significant at $P < 0.05$ and are expressed as mean ± SEM

Treatment of the diabetic rats significantly increased the bodyweight back to normal as also seen in glibenclamide treated group when compared to the untreated group. This finding aligns with the previous study conducted by [29]. However, the increase in bodyweight of the treated groups may be attributed to its shielding property in monitoring muscle wasting by reversing gluconeogenesis, lipolysis, glycogenolysis as well as appropriate control of glycemic status [30]. Furthermore, it could be suggested that there was a shift to carbohydrate as a source of energy with the preservation of proteins and fats which resulted in prevention of bodyweight decrease in diabetic rats treated with *Lantana camara* and glibenclamide. Serum creatinine, urea and uric acid of the untreated group 2 increased significantly when compared to the control group. This may be due to the deficiency or insensitivity of the insulin and subsequent inability of glucose to enter the extrahepatic tissues, thereby activating gluconeogenesis as an alternative route of glucose supply which further resulted to the generation of free glucogenic amino acids into the plasma [31]. These amino acids are then deaminated in the liver and then increase blood urea [32].

Creatinine is a metabolic intermediate of muscle creatine and its concentrations in blood is a factor of body muscle mass [33]. The increased level of creatinine in untreated diabetic group could be due to decreased body weight caused by muscle wasting as a result of the stimulation of lipolysis and proteolysis due to insulin defect [34]. The increase in uric acid concentrations observed in untreated group may follow the assertion of protein glycation which may also stimulate muscle wasting and increase the release of purine, the major source of uric acid. Administration of *Lantana camara* leaf extract decreased the levels of urea, creatinine and uric acid, regulated homeostatically glucose level and subsequently protect against renal impairment due to diabetes complications.

4. CONCLUSION

The study revealed that ethanol leaf extract of *Lantana camara* at a dose of 1000 mg/kg body weight possesses effective hypoglycemic and nephroprotective effects comparable to the chemotherapeutic antidiabetic drug (glibenclamide). However, pharmacological investigations are recommended to exactly

elucidate the mechanism of the observed hypoglycemic and nephroprotective potentials.

ETHICAL APPROVAL

The use of animals for the study was carried out in accordance with guidelines set by Institute for Laboratory Animal Research (ILAR) (2000).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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